AQUATIC ENTEROBACTERIACEAE

by

Moya Jones

Bacteriology Branch
Division of Laboratories
ONTARIO WATER RESOURCES COMMISSION

September, 1971

Introduction

In the summer of 1970, bacterial colonies were isolated from total plate count plates used to determine the total heterotroph population in fresh water lakes. In this study, described by the author (Jones, 1971) the identification of the Enterobacteriaceae was left at the familiar level of classification. The present study was undertaken to compare two procedures of Enterobacteriaceae identification and to determine if these characterization schemes could provide identification of the organisms at a generic level.

Methods

Following the identification of a plate count isolate as an Enterobacteriaceae, the organism was inoculated onto a fresh Trypticase-soy (T-soy, Difco) agar slant, incubated at 20°C for 48 hours, and then stored in screw capped tubes under refrigeration (approximately 4°C). The storage of these cultures lasted from two to three months. When cultures were removed from the refrigerator they were inoculated into T-soy broth and incubated at 37°C for 48 hours. Growing cultures were streaked onto T-soy agar slants and

amd

reincubated at 37° C for another 48 hours. From the slants an inoculum was streaked on MacConkey agar (Difco) plates to check the culture purity. Single well isolated colonies appearing on the plates after two days incubation at 37° C were streaked onto fresh T-soy slants. These slants were used as the inoculum source for identification tests.

Out of 403 Enterobacteriaceae cultures, 199 were lost in the processes of storage and recovery from refrigeration. Sixty-five cultures did not grow in the T-soy broth, 125 cultures died on transfer to the T-soy slants, and 9 cultures lost viability when streaked on MacConkey agar. On loss of viability, previous growth of the organism was reinoculated into fresh media but the viability was not recovered. Two hundred and four cultures were subjected to biochemical testing.

The Analytab system for the identification of Enterobacteriaceae was one of two series of biochemical characterization schemes used. This procedure is a commercially prepared series of twenty biochemical tests manufactured by Analytab Products Incorporated of New York. Pre-sterlized strips of small plastic capsules contain the dehydrated test media. Following inoculation the strips are incubated in a humid atmosphere within the provided sterile plastic containers, for 18 hours at 37° C.

Inocula were prepared by suspending the bacterial growth from T-soy slants in 4 mls of sterile buffered distilled water. The quantity of growth suspended approximated that of a 2 mm. colony. Sterile Pasteur pipettes were used to introduce the bacterial suspensions into the plastic capsules. In Table I, the twenty biochemical tests determined by the Analytab system are listed.

The second method of testing used the standard procedure of biochemical determinations by means of individual tubes of sterilized prepared media. The growth from T-soy slants rather than bacterial suspensions were used as the inocula. The tests were incubated at 37°C for 48 hours. The fifteen characterization tests carried out by this method are listed in Table I. A detailed description of reagents, media and test procedures used was presented by Bennett (1969).

ANALYTAB PROCEDURE	STANDARD PROCEDURE
Beta-galactosidase	lactose acidification
Arginine dihydrolase	arginine dihydrolase
lysine decarboxylase	lysine decarboxylase
ornithine decarboxylase	ornithine decarboxylase
citrate assimilation	citrate assimilation
H_2S production	H_2S production (in TSI)
urease	urease
tryptophane deamination	phenyl-alanine deamination
indole production	indole production
acetain production (V.P.)	acetain production (V.P.)
gelatin proteolysis	gelatin proteolysis
glucose acidification	glucose acidification
mannitol acidification	methyl-red reaction
inositol acidification	triple sugar reaction (TSI)
sorbitol acidification	motility
rhamnose acidification	
sucrose acidification	
melibiose acidification	
amygdaline acidification	
arabinose acidification	

Table I The biochemical tests for the characterization of Enterobacteriaceae used in the Analytab and standard testing procedures.

Results and Discussion

The Analytab and standard test procedures had eleven biochemical tests in common: the acidification of lactose and glucose, the dihydrolation of arginine, the decarboxylation of lysine and ornithine, the Voges-Proskauer reaction, the production of indol and hydrogen sulfide, the presence of urease, the utilization of citrate and gelatin protedysis. The results of these tests were compared for each of the 204 cultures and the number of discrepancies which occurred were recorded. In Figure I, the results of the comparison are presented. When different reactions resulted from the two procedures, the standard method result was noted. In this way, the discrepant results could be discussed in terms of the occurrence of positive reactions in one of the two methods. Table II shows the number of discrepant results which occurred for each of the eleven common tests, and the reactions determined by the standard method.

Citrate and lysine determinations gave the highest number of discrepancies between the standard and Analytab procedures. The citrate test determines if the organism can utilize citrate as the sole source of carbon. Both procedures used Simmons citrate test with bromthymol blue

as the pH indicator. The majority of discrepancies in the citrate test reactions occurred because the standard procedure gave positive results which the Analytab procedure failed to detect. The test for the decarboxylation of lysine also had high numbers of negative results by the Analytab method where positive reactions were determined by the standard tube technique. The pH indicator in the Analytab system was phenol red. The standard test procedure used bromcresol purple which has a much lower pH range than phenol red. Since the positive reaction for lysine decarboxylation is alkalinity of the medium, the lower pH range of the standard procedure medium explained the positive results recorded in this method and undetected by the Analytab method.

Arginine dihydrolase is an enzyme which allows the organism to convert arginine to urea and ornithine. The pH indicators for the positive alkaline reaction were again bromcresol purple for the standard procedure and phenol red for the Analytab procedure. The discrepancy in results between the two systems was attributed to the different pH indicators used. As in the case of lysine, the standard procedure gave positive arginine reactions where negative reactions occurred with the Analytab procedure.

Both test procedures for the detection of urease, an enzyme responsible for the degradation of urea, used the pH indicator phenol red. The Analytab test procedure failed to show enzyme activity already demonstrated by the standard procedure. This pattern of reaction accounted for most of the test result discrepancies.

The Voges-Proskauer test gave the second highest number of discrepancies between the two methods. This test detects the presence of acetylmethylcarbinol (acetoin) which is a product of carbohydrate metabolism. The two test procedures accounted for equal numbers of positive results in cases where discrepancies occurred.

The test for lactose fermentation involved two distinct procedures. In the standard method the substrate lactose is provided and its fermentation is indicated by acidification of the medium. The Analytab procedure tests for the presence of beta-galactosidase, an enzyme associated with lactose utilization. The method involves an enzyme inducer (isopropyl-thio-galactopyranoside) and the liberation of orthonitrophenol which gives a yellow colour, from the colourless substrate ONPG (orthonitrophenyl beta D galactopyranoside). Most of the result discrepancies were due to a positive beta-galactosi-

dase reaction (Analytab) where no lactose fermentation was detected in the standard procedure.

In the test for ornithine decarboxylase, the Analytab system accounted for more positive results than the standard method when discrepancies occurred. The same situation occurred in tests for the proteolysis of gelatin and the fermentation of glucose. The Analytab system gave positive results in these tests where negative reactions had been determined by the standard procedure.

In only one case did the test for hydrogen sulfide (H_2S) production give different results for the two test procedures. Both methods used thiosulfate as a precursor and iron salt as the reagent. One positive reaction was detected in the triple sugar iron agar (standard procedure) which did occur in the Analytab system. There were no discrepancies in the test for the presence of indol.

The test reactions determined by the standard procedures were compared to the identification scheme presented by Edwards and Ewing and revised by Ewing (1970). The Analytab reactions were compared with the scheme presented by Analytab which agrees with that of Ewing. Two additional sugar reactions,

the acidification of amygdaline and arabinose, and a test for tryptophan deaminase are included in the Analytab differentiation.

The Enterobacteriaceae cultures could not be identified on the basis of these schemes. The cultures differed from all recognized genera and species. For purposes of comparison with future work on the identification of Enterobacteriaceae the record of the isolated cultures' biochemical reactions is being kept.

Summary and Recommendations

The Analytab system results differed from those determined by standard procedures. This commercially prepared system cannot be used as a substituted procedure because of the result discrepancies.

Enterobacteriaceae isolated from lakes do not belong to the recognized genera and species of this family. The schemes of identification used to differentiate Enterobacteriaceae should be extended to include family members from the acquatic environment. This could be accomplished by means of extensive biochemical characterizations of acquatic Enterobacteriaceae and classification of the biochemical types by means of numerical taxonomy.

References

Bennett, E.A. 1969. A study of the distribution of Meterotrophic bacteria in the Great Lakes. Bacteriology Branch Interim Report, Division of Laboratories, Ontario Water Resources Commission.

Ewing, W.H. 1970. Difco Laboratories Publication, October 1970, 0320. Difco Laboratories, Detroit, Michigan.

Jones, M.T. 1970. The Georgian Bay Survey Bacteriology Report. Bacteriology Branch Interim Report, Division of Laboratories, Ontario Water Resources Commission.

Jones, M.T. 1971 A study of the taxonomic composition of bacterial populations in fresh-water lakes. Bacteriology Branch Interim Report, Division of Laboratories, Ontario Water Resources Commission.

TABLE II A DESCRIPTION OF TEST RESULT DIFFERENCES FROM THE STANDARD AND ANALYTAB PROCEDURES IN TERMS OF THE STANDARD PROCEDURE REACTIONS

TEST	NUMBER OF DIFFERENT REACTIONS	STANDARD PROCEDURE REACTIONS	
		Positive	Negative
CITRATE	87	61	26
LYSINE	87	59	28
V.P.	69	33	36
ARGININE	52	45	7
LACTOSE	43	11	32
ORNITHINE	19	8	11
UREASE	14	10	4
GELATIN	5	1	4
GLUCOSE	2		2
H_2S	1	1	
INDOLE	0		

FIGURE I: THE FREQUENCY OF DIFFERENT TEST RESULTS FROM THE STANDARD AND ANALYTAB TESTING PROCEDURES, PRESENTED AS PERCENTAGES OF THE TOTAL NUMBER OF INDIVIDUAL TESTS PERFORMED (204)